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TECHNIQUES IN THE STUDY OF POPULATION STRUCTURE IN PHILOTES SONORENSIS

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THE HANDLING OF LIVING MATERIAL has always been an important factor in the design of biological experiments. This factor is especially critical in studying population structure under natural conditions. One of the most accurate techniques for determining parameters of population structure is that of capturing, marking, releasing and subsequent recapturing of individuals (Ford 1951). Such experiments would be biased if a behavioral response is elicited or differential viability imposed as a result of the technique employed.

We have developed a handling technique in sampling populations of the small Lycaenid butterfly, *Philotes sonorensis,* over a period of three collecting years. This routine appears to have little or no effect on the subsequent behavior of the butterfly. The purpose of this report is to describe and evaluate the technique.

Our objectives were to describe the distribution, numbers, and movements of adult individuals of *P. sonorensis* within a small circumscribed area in the Fish Canyon portion of the San Gabriel Canyon Wash near Los Angeles. These individuals were classified as to 8 male and 5 female spot pattern phenotypes. (Figure 1). Six stations, each 80 meters in diameter, were set up and sampled in 1955 and 1956. These were separated by distances ranging from 96 to 433 meters from the center of one area to the perimeter of another. The stations were destroyed by trenching operations in 1961 because of water requirements. New experimental sites were established in 1963 in other areas of the wash.

Sampling was done as weather permitted during the flight period in March. In 1955 a total of 809 specimens were captured 1126 times during 9 collecting days over a 21 day period. In 1956 there were 972 specimens captured 1226 times for 11 collecting days over a 29 day period. Sampling was done between 9 A.M. and 3 P.M., one hour being allotted to each station during each day. The order in which the stations were collected on consecutive days was ramdomized in order to minimize possible differential effects correlated with time

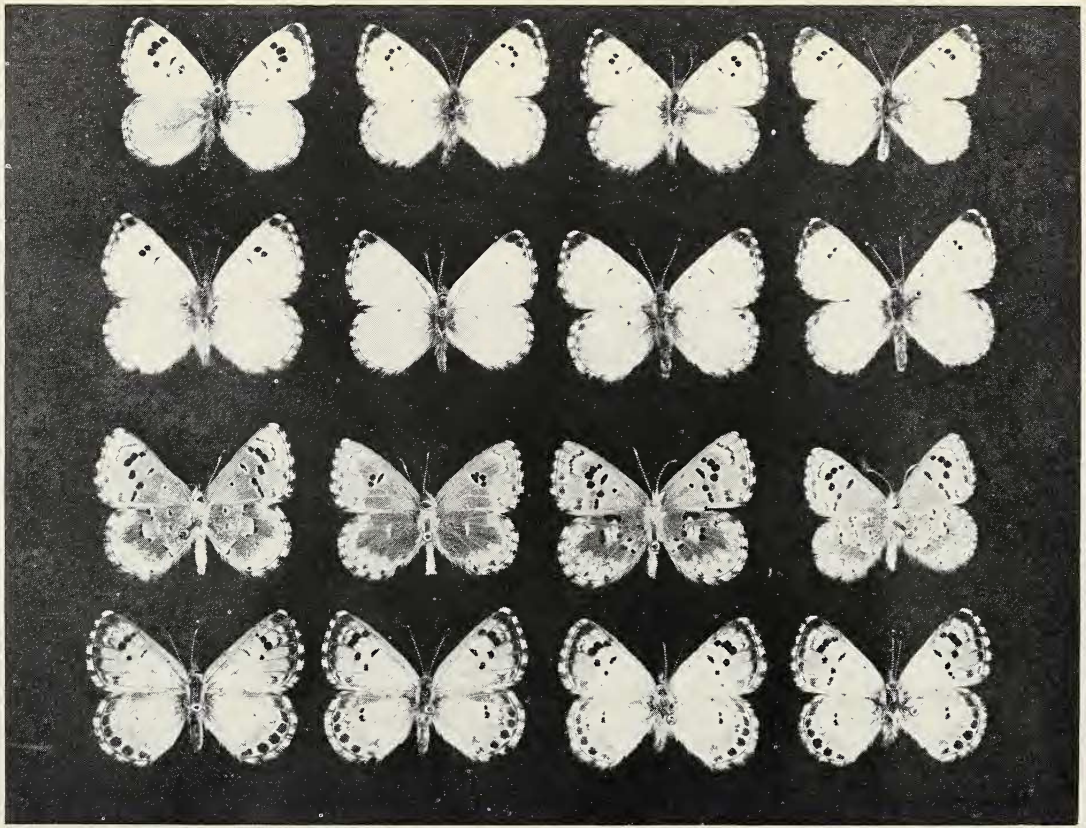
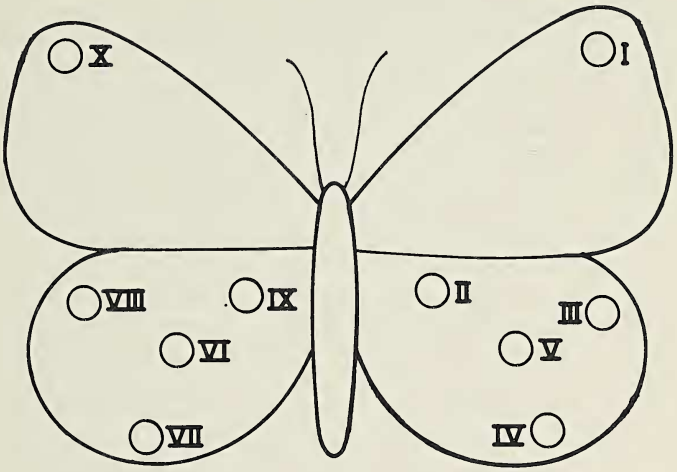


Fig. 1. Phenotypic classes found at San Gabriel which were recorded, the eight male types were classified according to the number and distribution of right forewing submarginal spots. Specimen 7 represents two classes; *comstocki,* which has the underside shown in specimen 10. Specimen 8 shows spotting assymmetry, classifiable as A on the right and D on the left side. Specimens 9 and 11 show two modal types of underside pattern, 12 the underside pattern of populations found outside the wash itself. Four female types were classified by the number of black spots found on the forewing cells of CUI and CU2. These range from 0 to 3 in specimens number 13 to 16 respectively. The female form of *comstocki* is not shown.

Fig. 2. (below) Photograph showing equipment described in the text. RHTM on right holding a specimen about to be placed in the recovery chamber on the portable desk. Vials with specimens can be seen in the compartment and the CO_2 tank behind. MSRS on left recording data.

Fig. 3. (above) Anaesthesis Tube.

of day. Each station was sampled for 30 minutes. This allowed 10 minutes for moving between stations and 20 minutes for handling. When a butterfly was caught it was removed from the net with a 1" x 4" shell vial and the vial was plugged with a cotton stopper. We avoided touching the specimens with anything other than the net and the vial. After the collecting period the vials of butterflies were assembled in the center of the collecting area. In all cases one of us (RHTM) classified and marked the specimens while the other (MSBS) recorded the data. This process took approximately 20 minutes per station. The equipment used is shown in Figure 2. This included a portable desk, carbon dioxide tank, and release carton. The compartment on the desk served to store vials in the shade. Each specimen was anesthetized in its vial by a 10 second exposure to CO_2 delivered at approximately 3 psi. The CO_2 was delivered from a small tank strapped to a pack frame for easy portability. A regulator maintained constant flow and pressure from the tank through the rubber tube to one of the glass tubes in a two hole rubber stopper inserted in the vial. The other glass tube in the stopper served as an exhaust to avoid excessive pressure in the vial (Figure 3). The anaesthetized butterfly was removed from the vial with flat bladed insect forceps and classified according to sex, forewing spot pattern and the area and date of previous capture if it had been a recaptured specimen. The specimen was then marked to indicate the date and area of capture. This was done by putting a dot of "Pactra" lacquer on the wing underside. The critical factor of this operation was maintaining a proper paint consistency. This was done by trial and error, using acetone as a diluent with a blunted dissecting needle. Six different colors were used to denote the six stations. These were applied to one of ten distinct underwing areas to denote the date of capture (Figure 4). The butterfly was then carefully laid on the bottom of a one gallon ice cream carton and allowed to recover. Recovery time varied, but seldom exceeded two minutes. The process was then repeated. After handling the last specimen, all the gear was assembled. Just prior to moving to the next station the carton was held upright, facing the sun, and was gently tapped so that the remaining specimens would fly off. If any specimen remained, the carton was inverted and vigorously tapped. If a specimen was not able to fly "normally" a distance of 10 feet, it was removed from the population and the event recorded. After the first day's collection, subsequent collecting within about 10 feet of the center of the station was avoided as a precaution again recapturing injured specimens, if any should exist. In 1955, 21 individuals including 10 recaptures, and in 1956, 37 individuals including 8 recaptures, were removed from the population. These figures are not wholly indicative of the effectiveness of the technique since about half of these represented specimens sampled for study.



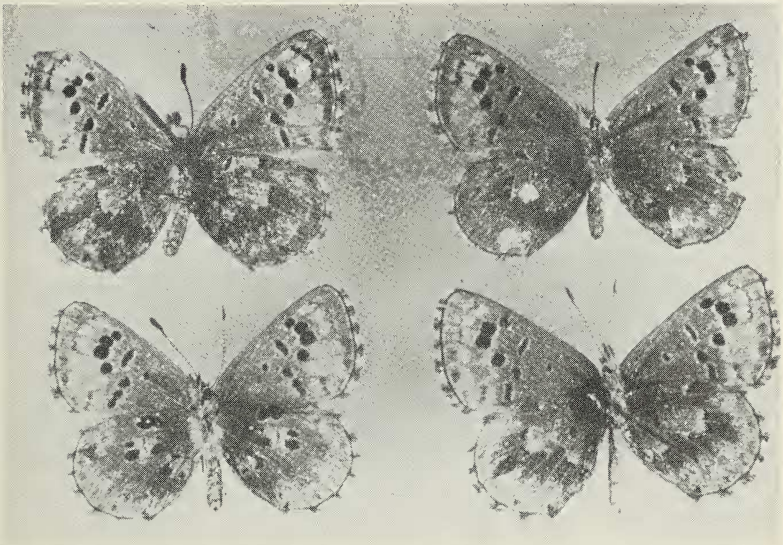


Fig. 4. Marking scheme on underwings to denote data of capture. The circles indicate the positions of the lacquer marks on the wings which correspond to the collecting day. (designated by Roman numerals).

Fig. 5. Recaptured specimens sampled on the last collecting day of 1955.

The distinctive feature of our technique is the use of CO_2 anaesthesis in the field. We feel that anaesthesis greatly decreases the probability of damage due to handling, especially when examining and marking such a small butterfly as *P. sonorensis*. The procedure was adopted because of its general acceptability in the laboratory for research in insect physiology and genetics. For example, Seiger (1953) studied the effects of different anaesthetics on the dipteran, *Drosophila mulleri*. He found no significant difference in fertility and fecundity between flies which had been given no anaesthesis and flies which had been anaesthetized with CO_2. The average quiescent period after anaesthesis was one minute. This was sufficient time to classify an individual for sex and wing pattern, determine if it had been recaptured, mark the lower wings, and allow the lacquer to dry. Following recovery, the individuals tended to remain quietly in the container, thus further minimizing damage. The lacquer fastens the scales to the wing membrane and dries so quickly that no more than ten losses were suffered by insects sticking to the container or themselves. In the laboratory, *P. sonorensis* could withstand CO_2 anaesthesis in excess of 10 minutes with no apparent ill effects, although under longer periods of exposure, partial paralysis would occur and eventually death ensue. There was no evidence for cumulative effect of repeated exposures of 10-30 seconds duration to CO_2. The possibility that abnormal behavior might result from anaesthesis has not been fully explored. In the first flight after anaesthesia, there appears to be a tendency for the butterflies to exhibit an escape behavior. After alighting once the behavior is not apparent. There appears to be no difference between the behavior of a butterfly in its first flight after anaesthesis and the behavior of a butterfly in flight after being captured in a net and released without anaesthesis. Although we feel that the advantages of anaesthesia far outweigh any possible disadvantages, we plan to determine whether there are any real effects of CO_2 on the behavior of *P. sonorensis* in future experiments.

We believe that the most important evidence of the negligible effect of our overall technique on behavior lies in the consistency of our data for two years with respect to the highly non-random pattern of movement. Another evidence was the remarkable behavior of 5 individuals in 1956. These moved away from their area of capture and subsequently returned. There are several reasons which indicate that viability effects are also negligible. Figure 5 shows four marked specimens sampled on the last day of 1955. These appear to be quite undamaged, that at the upper right having been followed for eleven days and caught four times. In the course of our studies, several insects flew the distance between the two farthest stations (433 meters), one flying a minimum of 819 meters. Lastly, because many specimens were captured more than once, it is possible to infer whether multiple handling had an additive effect on viability by com-

paring observed with expected values of multiple recaptures 0, 1, 2, 3 and 4 times.

Using a poisson distribution, the data for 1955 gives $\chi^2 = 23.7$ and $P = < .0001$ for 3 df; and for 1956, $\chi^2 = 5.4$ and $P = .12$ for 3 df. The highly significant departure from expected in 1955 is due entirely to an excess of specimens recaptured 3 and 4 times. The meaning of this departure is obscure, although it does not contradict the hypothesis of additive deleterious handling effects.

We found that the marking technique enabled us to distinguish individuals without confusion. The method can be compared to a punch card system in which each butterfly carries a recorded code on its wings, the color of the spot indicating the station where each capture took place and the position of the spot on he wing indicating the date of each capture. Thus the population size for each station can be determined for any given collecting day (Dowdeswell, Fisher and Ford 1949, Ford 1951), patterns of movement can be discerned and the life span of individuals can be calculated. These characteristics can be further quantified by correlation with maculation type and sex of the individuals. These data and conclusions are being prepared for publication in this journal.

For comparative information on field techniques in population study with Lepidoptera, the reader is referred to Abbot (1959), Ehrlich and Davidson (1960), Evans (1955), and Fales (1959); as well as those of Ford and his associates (op. cit.) We feel our technique has the least effect on viability and behavior differentials, particularly with reference to the use of anaesthesis; minimizing number of identifying marks on each individual; limiting individual contact to net, vial and forceps; and no more than 30 minute retention in the field. The last item, of course, is part of our experimental design. We feel that all such studies should take this factor into account, in spite of statistical difficulties in treating same day recaptures.

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SUMMARY

1. A method for anaesthetizing and marking individuals in order to determine population structure is described.

2. The benefits and possible disadvantages of the method are examined and some applications of the technique are mentioned.

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